

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ZERO-ORDER APPROACHES FOR TRICLABENDAZOLE ESTIMATION IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, and precise zero order derivative UV spectroscopic method has been devised and validated for the estimation of triclabendazole in bulk and tablet dosage form. According to Beer's law, the concentration of triclabendazole in methanol ranges from 2 to 12 µg/ml, and its absorbance reaches its maximum at 306 nm. The area under the curve in absorption spectra is measured between 301 and 311 nm. The regression coefficient (r^2) was found to be 0.995 following a linearity investigation, demonstrating acceptable linearity and precision over this concentration range. The percentage recovery was found to be 99.5%, 99%, and 103.3%, while the limits of detection (LOD) and quantitation (LOQ) were found to be 1.55 and 4.71 micrograms per milliliter, respectively. Furthermore, excellent precision was indicated by the methodologies' relative standard deviation (% RSD) values, which were less than 2%. All validation metrics, including linearity, accuracy, precision,

ruggedness, LOD, and LOQ, were assessed in accordance with ICH requirements. The developed and proven method can be used to regularly estimate the dosage of triclabendazole

in both bulk and tablet form.

KEYWORDS: Triclabendazole; Quantification; Validation; RP-HPLC; HPTLC, Anthelmintic.

INTRODUCTION

Triclabendazole is a benzimidazole derivative that is mostly used as an anthelmintic medication. It works particularly well against liver flukes, or *Fasciola hepatica*. It functions by interfering with parasites' ability to use microtubules. Reliable analytical techniques for its quantification and quality control are essential because of its clinical significance, particularly in parasite infections in humans and animals. In contrast to other trematocidal medications, triclabendazole (TCBZ, 6-chloro-5(2,3-dichlorophenoxy)-2-methylthio-benzimidazole), a halogenated benzimidazole (BZD) thiol derivative, exhibits high efficacy against both the immature and mature stages of *Fasciola hepatica* in sheep and cattle.^[2] Intestinal nematode infections are one of the main causes of financial losses in the sheep breeding industry. As the prevalence of parasite resistance continues to rise, veterinarians are now using a range of anthelmintic medications in combination to treat animals quickly.^[3] Triclabendazole dissolves readily in organic solvents like acetonitrile and methanol but is essentially insoluble in water. Methanol is frequently chosen as a solvent for UV spectrophotometric measurement because of its solubility properties. Achieving clear spectra, a well-shaped peak, and repeatable absorbance values depends heavily on the choice of solvent.

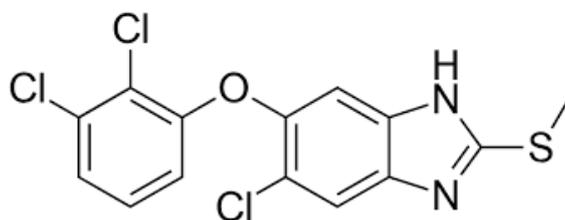


Figure 1: Chemical Structure of Triclabendazole.

Few analytical methods using UV, HPLC, RP-HPLC, and HPTLC have been described for determining Triclabendazole in pure medication and pharmaceutical dosage forms, according to review of the literature. The current endeavor attempts to create and validate a novel, fast, simple, precise, and specific Zero order derivative UV Spectrophotometric method for estimating Triclabendazole in tablet and bulk dose form.

MATERIALS AND METHODS

Instrument

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals

Triclabendazole pure drug gift sample was given by Ce-Chem Pharmaceuticals Pvt Ltd and its pharmaceutical dosage Triclabendazole 20 tablets obtained from Medsuvac Lifesciences.

Solvent

Methanol is used as a solvent.

Selection of analytical wavelength

Appropriate dilutions of Triclabendazole were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400 nm. The absorption spectra obtained and show maximum absorbance at 306 nm, as the wavelength for detection.

Preparation of standard stock solution

100mg of Triclabendazole was weighed accurately transferred into 100 ml of volumetric flask and diluted in Methanol upto the mark. From this, the solution was further diluted into 100 μ g/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml into 10 ml individual volumetric flask and diluted in Methanol up to the mark, this gives 2, 4, 6, 8, 10 and 12 μ g/ml concentration.

Preparation of sample solution

20 tablets of Triclabendazole marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Triclabendazole was transferred into 100ml volumetric flask then it was diluted with Methanol and make upto the mark.

METHOD AND VALIDATION

The method was validated according to the ICH guidelines.

RESULT AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

Linearity shows how well the response of the method changes in proportion to the concentration of the drug within a given range. In other words, when the concentration increases, the absorbance should also increase in a consistent and predictable manner. The linearity was established in the range of 2-12 $\mu\text{g/ml}$ was measured at 306nm and absorbance values are shown in table 1. The calibration curve was prepared by plotting graph against the concentration vs absorbance and therefore the graph shown in Fig-3 statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision

Precision indicates how close the results are when the same sample is tested multiple times under similar conditions. If the variation between repeated measurements is very small, the method is considered precise. Precision was established by intra-day and inter-day was determined by analysing the same concentration for six times in a same day. Inter-day precision was analysing the same concentration daily for six days shown in table-3.

Accuracy

The accuracy of an analytical method says that closeness of test results obtained by that method of the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels at 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table -4.

Ruggedness

Ruggedness evaluates whether the method gives consistent results when small changes are made, such as using different analysts or performing the test on different days. A rugged method produces similar results despite these minor variations. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table-5)

LOD and LOQ

LOD is the smallest amount of drug that the method can detect, even if it cannot measure it accurately. LOQ is the lowest amount of drug that can be measured accurately and precisely using the developed method. LOD and LOQ were calculated by using following formula

$$\text{LOD} = 3.3(\sigma/S) \text{ and } \text{LOQ} = 10 (\sigma / S)$$

LOD and LOQ value of Triclabendzole were found be 1.55 $\mu\text{g/mL}$ and 4.71 $\mu\text{g/mL}$.

Table 1: Results of calibration curve at 306nm by zero order derivative spectroscopy.

Sl No	Concentration in $\mu\text{g/ml}$	Absorbance \pm Standard deviation
1	0	0
2	2	0.183 \pm 0.0018
3	4	0.273 \pm 0.0014
4	6	0.354 \pm 0.0013
5	8	0.503 \pm 0.0014
6	10	0.642 \pm 0.0014
7	12	0.731 \pm 0.0014

* Average of six determinations

Table 2: Regression parameters of Triclabendazole by Zero order spectroscopy.

Regression	Parameter Results
Range	2-12 $\mu\text{g/ml}$
λ_{max}	306nm
Regression equation	$y = 0.0596x + 0.026$
Slope(b)	0.0596
Intercept (a)	0.026
Correlation coefficient	0.995
Sandell's sensitivity	0.0175
LOD($\mu\text{g/ml}$)	1.55 $\mu\text{g/ml}$
LOQ($\mu\text{g/ml}$)	4.71 $\mu\text{g/ml}$

$$Y = bx + a^{**}$$

Table 3: Determination of Precision results for Triclabendazole at 306nm by Zero order spectroscopy.

Concentration ($\mu\text{g/ml}$)	Intra-day Absorbance \pm Standard deviation*	% RSD	Inter-day Absorbance \pm Standard deviation*	% RSD
2	0.183 \pm 0.0018	1.019	0.172 \pm 0.0008	0.473
4	0.274 \pm 0.0014	0.516	0.268 \pm 0.0019	0.732
6	0.354 \pm 0.0013	0.385	0.358 \pm 0.0005	0.152
8	0.503 \pm 0.0014	0.281	0.505 \pm 0.0005	0.102
10	0.642 \pm 0.0014	0.22	0.653 \pm 0.001	0.193
12	0.731 \pm 0.0014	0.193	0.745 \pm 0.0005	0.069

* Average of six determinations, ** Percentage relative standard deviation.

Table 4: Determination of accuracy results for Triclabendazole at 306nm by Zero order spectroscopy.

Spiked levels	Amount of sample ($\mu\text{g/ml}$)	Amount of standard ($\mu\text{g/ml}$)	Amount recovered	% Recovery \pm Standard deviation*	%RSD**
50	6	3	8.95	99.5	0.0605
10	6	6	11.88	99	0.149
150	6	9	15.49	103.3	0.094

* Average of six determinations, ** Percentage relative standard deviation.

Table 5: Determination of ruggedness results of Triclabendazole at 306nm by Zero order spectroscopy.

Analysts	Day-1	Day-2
Mean absorbance	0.354	0.358
\pm Standard deviation*	0.0013	0.0005
%RSD**	0.385	0.152

* Average of six determinations, ** Percentage relative standard deviation.

FIGURES

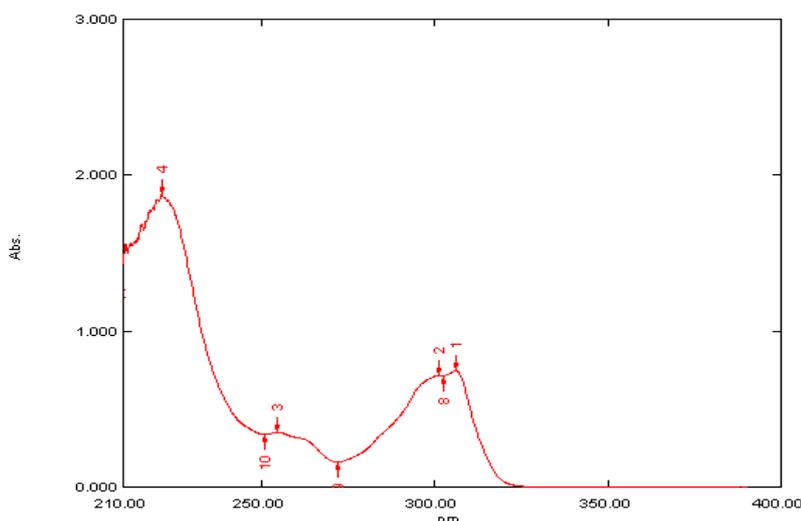


Fig. 2: Zero order spectrum of Triclabendazole at 306nm.

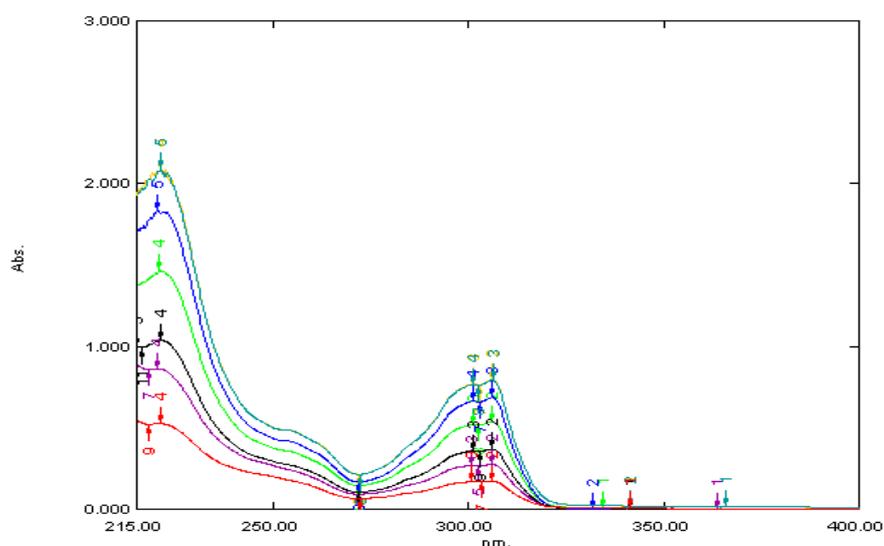


Figure 3: Zero Order Overlain Spectra of Triclabendazole Showing Absorbance At 306nm.

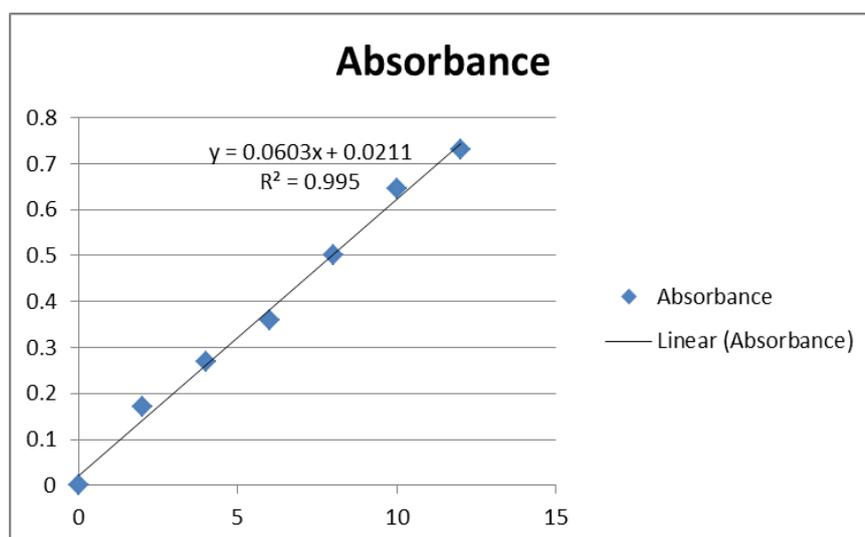


Figure 4: Calibration curve of Triclabendazole by Zero order derivative spectroscopy.

CONCLUSION

The analytical method developed for Triclabendazole was validated as per ICH guidelines demonstrating simplicity, specificity, accuracy, economy, and sensitivity. This method is suitable for regular analysis of Triclabendazole in both bulk form and pharmaceutical preparations.

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